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FORM PTO-1390 (REV 5-93)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY DOCKET NO. 100564-00095	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				DATE: January 18, 2002	
				U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.5) Not Yet Assigned <b>10/031401</b>	
INTERNATIONAL APPLICATION NO. PCT/EP00/06905		INTERNATIONAL FILING DATE 19 July 2000		PRIORITY DATE CLAIMED 19 July 1999	
TITLE OF INVENTION: CYCLIC PEPTIDOMIMETIC UROKINASE RECEPTOR ANTAGONISTS					
APPLICANT(S) FOR DO/EO/US: WILHELM, Olaf; KEBLER, Horst; BÜRGLE, Markus; POTTHOFF, Nils; SCHMIEDEBERG, Niko					
<p>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371. (THE BASIC FILING FEE IS ATTACHED)</p> <p>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input type="checkbox"/> This express request to begin national examination procedures [35 U.S.C. 371(f)] at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input type="checkbox"/> A proper demand for International Preliminary Amendment was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed [35 U.S.C. 371(c)(2)]</p> <p>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</p> <p>6. <input checked="" type="checkbox"/> A translation of the International Application into English [35 U.S.C. 371(c)(2)].</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 [35 U.S.C. 371(c)(3)]</p> <p>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input type="checkbox"/> have been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input checked="" type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 [35 U.S.C. 371(c)(3)].</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) [35 U.S.C. 371(c)(4)].</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C. 371(c)(5)].</p> <p>Items 11 - 16 below concern other document(s) or information included:</p> <p>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.</p> <p>12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.  <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information: <input checked="" type="checkbox"/> PCT/ISA/210; International Application as originally filed; PCT/IPEA/416 (in German); PCT/IPEA/409 (in German); 4 sheets of Amended Claims Drawings (12 sheets)</p>					

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**PATENT APPLICATION**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:  
Olaf WILHELM et al.

Appln. No.: PCT/EP00/06905

Filed: Concurrently herewith

Attorney Dkt. No.: 100564-00095

For: CYCLIC PEPTIDOMIMETIC UROKINASE RECEPTOR ANTAGONIST

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

January 18, 2002

Sir:

Prior to calculation of the filing fees and initial examination of the application, please amend the above-identified application as follows:

**IN THE CLAIMS:**

Please amend claims 5, 7, 8, 10-13 as follows:


5. (Amended) The compound as claimed in claim 1, characterized in that at least 2 of the amino acid residues  $X^{22}$ ,  $X^{23}$ ,  $X^{24}$ ,  $X^{25}$ ,  $X^{26}$ ,  $X^{27}$ ,  $X^{28}$  and  $X^{30}$  have the same side chain as an amino acid at the same position in the native uPA sequence.
7. (Amended) A pharmaceutical composition, which contains as active substance at least one compound as claimed in claim 1, where appropriate together with pharmaceutically common carries, excipients or diluents.
8. (Amended) The use of a compound as claimed in claim 1 for preparing a uPAR antagonist.

10. (Amended) The use of a command as claimed in claim 1 for preparing a targeting vehicle for cells expressing uPAR.
11. (Amended) The use of a compound as claimed in claim 1 for preparing an angiogenesis inhibitor.
12. (Amended) The use of a compound as claimed in claim 1 for preparing a targeting vehicle for cells expressing uPAR.
13. (Amended) The use of a compound as claimed in claim 1 for preparing an angiogenesis inhibitor.

### REMARKS

Claims 1-13 are pending in this application. By this Amendment, claims 5, 7, 8, 10-13 are amended to correct the multiple dependency thereof and to place this application into better condition for examination. No new matter is added.

Respectfully submitted,

  
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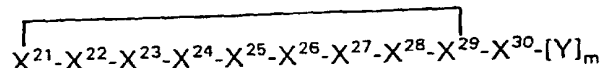
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EP0006905

# Claims

5

1. A compound of the general structural formula (I):



10

wherein  $X^{21}-X^{30}$  are monomeric building blocks, preferably aminocarboxylic acid residues and are derived from a structure in which  $X^{21}$  = D-Cys,  $X^{22}$  = Asn,  $X^{23}$  = Lys,  $X^{24}$  = Tyr,  $X^{25}$  = Phe,  $X^{26}$  = Ser,  $X^{27}$  = Asn,  $X^{28}$  = Ile,  $X^{29}$  = Cys and  $X^{30}$  = Trp, Y is a spacer and m is 0 or 1, and the monomeric building blocks are linked via  $-\text{CONR}^1$  or  $-\text{NR}^1\text{CO}$  bonds, in which  $R^1$  in each case independently is hydrogen, methyl or ethyl, and pharmaceutically acceptable salts and derivatives thereof,

15

with the proviso that at least one of the amino acid residues  $X^{21}-X^{30}$  of the lead structure is replaced by one of the amino acid residues listed below:

20

25

$X^{21}$ : Asp, Glu, 2,3-diaminopropionic acid (Dap),  
 2,4-diaminobutyric acid (Dab),  
 D-penicillanine (D-Pen), allylglycine (Alg),  
 ornithine (Orn), Lys;

$X^{22}$ : Asp, Glu;

30

$X^{23}$ : Dab, Dap, His, citrulline (Cit),  
 homocitrulline (Hci), norleucine (Nle);

$X^{24}$ : homophenylalanine (Hph), 1,2,3,4-  
 tetrahydroisoquinoline-3-carboxylic acid  
 (Tic), thienylalanine (Thi), Trp,  
 phenylglycin (Phg), 1-naphthylalanine (1-  
 Nal), 2-naphthylalanine (2-Nal), Cha  
 (cyclohexylalanine);

35

$X^{25}$ : Trp, Tic, Thi, Hph, Phg;

AMENDED SHEET

26-07-2001

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X<sup>26</sup>: Val;X<sup>27</sup>: Asp, Glu;X<sup>28</sup>: Cha, 2-aminobutyric acid (Abu), tert-leucine  
(Tle),  $\alpha$ -aminoisobutyric acid (Aib);5 X<sup>29</sup>: Asp, Glu, Dap, Dab, Alg, D-Pen, Orn, Lys;

2. The compound as claimed in claim 1,  
**characterized in that**

10 at least one of the amino acid residues X<sup>21</sup>-X<sup>30</sup> of  
the lead structure has one of the meanings listed  
below:

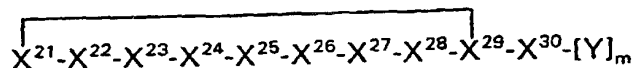
X<sup>21</sup>: D-Pen;X<sup>23</sup>: Dap, Dab, Cit, Hci, Nle, His;X<sup>24</sup>: Thi, Hph, Phg, 1-Nal, 2-Nal, Cha;15 X<sup>25</sup>: Thi;X<sup>27</sup>: Asp;X<sup>28</sup>: Val, Cha.

3. The compound as claimed in claim 1,  
**characterized in that**

20 at least one of the amino acid residues X<sup>21</sup>-X<sup>30</sup> of  
the lead structure has one of the meanings listed  
below:

X<sup>21</sup>: D-Pen;25 X<sup>23</sup>: Dab, Nle, Cit, Hci;X<sup>24</sup>: 1-Nal, 2-Nal, Cha;X<sup>25</sup>: Thi;X<sup>28</sup>: Cha.

- 30 4. A compound of the general structural formula (I):



35 wherein X<sup>21</sup>-X<sup>30</sup> are monomeric building blocks,  
preferably aminocarboxylic acid residues and are  
derived from a structure in which X<sup>21</sup> = D-Cys, X<sup>22</sup>  
= Asn, X<sup>23</sup> = Dap, Dab or Nle, X<sup>24</sup> = Tyr, X<sup>25</sup> = Phe,

X<sup>26</sup> = Ser, X<sup>27</sup> = Asn, X<sup>28</sup> = Ile, X<sup>29</sup> = Cys and X<sup>30</sup> = Trp, Y is a spacer and m is 0 or 1, and the monomeric building blocks are linked via -CONR<sup>1</sup> or -NR<sup>1</sup>CO bonds, in which R<sup>1</sup> in each case independently is hydrogen, methyl or ethyl, and pharmaceutically acceptable salts and derivatives thereof.

5. The compound as claimed in *claim 1* [any of the preceding claims]

**characterized in that**

at least 2 of the amino acid residues X<sup>22</sup>, X<sup>23</sup>, X<sup>24</sup>, X<sup>25</sup>, X<sup>26</sup>, X<sup>27</sup>, X<sup>28</sup> and X<sup>30</sup> have the same side chain as an amino acid at the same position in the native uPA sequence.

6. The compound as claimed in claim 5, **characterized in that**

at least 2 of the amino acid residues X<sup>24</sup>, X<sup>25</sup>, X<sup>28</sup> and X<sup>30</sup> have the same side chain as in the native uPA sequence.

7. A pharmaceutical composition, which contains as active substance at least one compound as claimed in *claim 1* [any of claims 1 to 6] where appropriate together with pharmaceutically common carriers, excipients or diluents.

8. The use of a compound as claimed in *claim 1* [any of claims 1 to 6] for preparing a uPAR antagonist.

9. The use as claimed in claim 8 for controlling disorders associated with uPAR expression, in particular for controlling tumors.

10. The use of a compound as claimed in *claim 1* [any of claims 1 to 6] for preparing a targeting vehicle for cells expressing uPAR.







12/1/97

Cyclic peptidomimetic urokinase receptor antagonists

**Description**

5 The present invention relates to cyclic peptides as  
inhibitors of urokinase binding to the urokinase  
receptor, which are suitable as pharmaceutical active  
substances for disorders mediated by urokinase and its  
10 receptor. The substances of the invention are peptides  
derived from the uPA sequence and display, as ligands  
of the urokinase receptor (uPAR), an antagonistic  
action and are denoted uPAR antagonists hereinbelow.

The serine protease uPA (urokinase-type plasminogen  
15 activator) is responsible for various physiological and  
pathological processes, such as, for example,  
proteolytic degradation of extracellular matrix  
material which is required for the invasiveness and  
migration of the cells and also for tissue remodeling.  
20 uPA binds with high affinity ( $K_D = 10^{-10}$ - $10^{-9}$ M) to the  
membrane-bound uPA receptor (uPAR) on the cell surface.

The binding of uPA to its receptor is involved in many  
invasive biological processes such as, for example,  
25 metastasis of malignant tumors, implantation of  
trophoblasts, inflammations and angiogenesis.  
Therefore, uPAR antagonists are capable of inhibiting  
the invasiveness, metastasis and angiogenesis of  
tumors. uPAR antagonists may be employed as agents for  
30 the therapy of invasive and metastasizing cancers in  
which uPA and uPAR appear at the invasive foci of  
tumors (Dano et al.: The receptor for urokinase  
plasminogen activator: Stromal cell involvement in  
extracellular proteolysis during cancer invasion, in:  
35 Proteolysis and Protein Turnover, Barrett, A.J. and  
Bond, J., HRSB, Portland Press, London, 1994, 239), for  
example in cancers of the breast, lung, intestine and  
ovaries. Moreover, uPAR antagonists may also be  
employed for other purposes in which inhibition of the  
40 proteolytic activation of plasminogen is required, for

example for controlling disorders such as arthritis, inflammations, osteoporosis, retinopathies and for contraception.

- 5 The uPA receptor is described in WO 90/12091 and also in the publications Ploug et al., J. Biol. Chem. 268 (1993), 17539 and Ronne et al., J. Immunol. Methods 167 (1994), 91.
- 10 uPA is synthesized as single-chain molecule (pro-uPA) and converted enzymically into an active two-chain uPA. The uPA molecule consists of three structurally independent domains, the N-terminally located growth factor-like domain (GFD, uPA 1-46), a ring structural
- 15 domain (uPA 45-135) and the serine protease domain (uPA 159-141). GFD and the ring domain together form the "amino-terminal" fragment of uPA (ATF, uPA 1-135) which is generated by further proteolytic cleavage of two-chain uPA. ATF binds to the uPA receptor with a similar
- 20 affinity as uPA.

- The receptor-binding region of uPA extends across the region of amino acids 12 to 32, since a peptide containing the uPA amino acid residues 12 to 32 (with
- 25 cysteine at position 19 being replaced by alanine) competes with ATF for binding to the uPA receptor (Appella et al., J. Biol. Chem. 161 (1987), 4437-4440). This study furthermore showed that said peptide, even after cyclization due to bridging of the two cysteine
- 30 residues at positions 12 and 32, displayed an affinity for the uPA receptor. In an alternative approach, Goodson et al., (Proc. Natl. Acad. USA 91 (1994), 7129-7133) identified antagonistic uPA peptides for the uPAR by means of screening a bacteriophage peptide library.
- 35 These peptides showed no obvious sequence homology to the natural uPAR-binding uPA sequence. More recent publications (Rettenberger et al., Biol. Chem. Hoppe-Seyler 376 (1995), 587-594); Magdolen et al., Eur. J. Biochem. 237 (1996), 743-751; Goretzki et al.,

Fibrinolysis and Proteolysis 11 (1997), 11-19) describe further studies on the uPAR binding region of uPA. In this connection, the residues Cys19, Lys23, Tyr24, Phe25, Ile28, Trp30 and Cys31 were identified as  
5 important determinants for uPA/uPAR interaction. Said studies identified a uPA peptide with the uPA amino acids 16 to 32 as the most active inhibitor.

Magdolen et al. (1996) supra analyze the uPAR binding  
10 region of the uPA molecule by using a peptide with the uPA amino acids 14 to 32 and peptides derived therefrom. However, these peptides and also peptides used by other groups (cf. e.g. Appella et al. (1987) supra) have a relatively low affinity for uPAR.

15 WO-A-94/22646 discloses linear peptides having a length of 6 to 18 amino acids which come from the region of the uPA amino acids 14 to 33. This study describes short peptides derived from uPA (uPA 21-29 and uPA  
20 21-26) being capable of influencing the growth of keratinocytes. Although WO-A-94/22646 indicates a possible use of the claimed peptides for blocking uPA/uPAR interaction, no data or indications of such binding studies whatsoever are shown. Moreover, the  
25 linear peptides uPA 21-29 and uPA 21-26, denoted "preferred", do not contain the minimal uPAR binding region of linear uPA peptides, which includes the sequence region of amino acids 19 to 31. Thus, the influence of said short peptides on keratinocyte growth  
30 is most probably not based on a uPA/uPAR interaction.

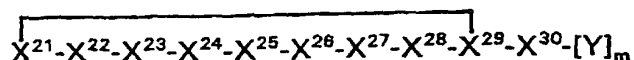
WO 98/46632 discloses uPAR peptide inhibitors which are derived from the linear peptide uPA (19-31) and cyclic derivatives thereof and which carry D-amino acid  
35 residues in selected positions.

An example of a peptide inhibitor of this kind is the peptide cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]uPA<sub>21-30</sub>. This peptide already has quite high affinity for uPAR (IC<sub>50</sub>=78nM)

which is only 4 times lower than the affinity of the amino-terminal fragment of uPA (ATF = amino acids 1-135 of urokinase) which has an  $IC_{50}$  of 21 nM. The corresponding peptide composed exclusively of L-amino acids, cyclo[21,29]-[Cys<sup>21</sup>,Cys<sup>29</sup>]-uPA<sub>21-30</sub>, has a hundred-fold lower activity compared with ATF.

It was the object of the present invention to modify the structure of the uPAR peptide inhibitor by incorporating isostructural or/and isofunctional natural and non-natural amino acids and thus to achieve further improvement regarding the affinity for uPAR, serum stability or/and therapeutic action.

The present invention thus relates to compounds of the general structural formula (I):



wherein

$X^{21}-X^{30}$  are monomeric building blocks, preferably aminocarboxylic acid residues and are derived from a structure in which  $X^{21}$  = D-Cys,  $X^{22}$  = Asn,  $X^{23}$  = Lys,  $X^{24}$  = Tyr,  $X^{25}$  = Phe,  $X^{26}$  = Ser,  $X^{27}$  = Asn,  $X^{28}$  = Ile,  $X^{29}$  = Cys and  $X^{30}$  = Trp,

Y is a spacer and m is 0 or 1, and the monomeric building blocks are linked via  $-\text{CONR}^1$  or  $-\text{NR}^1\text{CO}$  bonds, in which  $R^1$  in each case independently is hydrogen, methyl or ethyl, and to pharmaceutically acceptable salts and derivatives thereof,

with the proviso that at least one of the amino acid residues  $X^{21}-X^{30}$  of the lead structure is replaced by one of the amino acid residues listed below:

$X^{21}$ : Asp, Glu, 2,3-diaminopropionic acid (Dap), 2,4-diaminobutyric acid (Dab), penicillamine (Pen), D-Pen, allylglycine (Alg), ornithine (Orn), Lys;  
 $X^{22}$ : Gln, Asp, Glu;

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- X<sup>23</sup>: Orn, Dap, Arg, His, citrulline (Cit),  
homocitrulline (Hci), norleucine (Nle);
- X<sup>24</sup>: Phe, homophenylalanine (Hph), 1,2,3,4-  
tetrahydroisoquinoline-3-carboxylic acid (Tic),  
thienylalanine (Thi), Trp, phenylglycin (Phg), 1-  
naphthylalanine (1-Nal), 2-naphthylalanine (2-  
Nal), Cha (cyclohexylalanine);
- X<sup>25</sup>: Tyr, Trp, Tic, Thi, Hph, Phg;
- X<sup>26</sup>: Thr, Val, homoserine (Hse);
- X<sup>27</sup>: Gln, Asp, Glu;
- X<sup>28</sup>: Val, Leu, 2-aminobutyric acid (Abu), tert-leucine  
(Tle), norvaline (Nva), Nle, α-aminoisobutyric  
acid (Aib), Cha;
- X<sup>29</sup>: Asp, Glu, Dap, Dab, Alg, Pen, D-Pen, Orn, Lys;
- X<sup>30</sup>: Thi, Phe, Tyr, 2-Nal, 1-Nal, octahydroindolyl-2-  
carboxylic acid (Oic), His, thiazolylalanine  
(Thia), Phg, tryptamine, tryptophanamide (Trp-  
NH<sub>2</sub>).

20 Preference is given to peptides in which at least one  
of the amino acid residues X<sup>21</sup>-X<sup>30</sup> of the lead structure  
has one of the meanings listed below:

- ```

X21: D-Pen;
X22: Gln;
25 X23: Orn, Dap, Dab, Arg, Cit, Hci, Nle, His;
X24: Phe, Thi, Hph, Phg, 1-Nal, 2-Nal, Cha;
X25: Thi;
X27: Asp;
X28: Nle, Val, Cha;
30 X29: Pen;
X30: Phe, Thi, Tyr, Oic, 1-Nal, Hph, Thia, Trp-
NH2.

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Particular preference is given to peptides in which at  
35 least one of the amino acid residues  $X^{21}-X^{30}$  of the lead  
structure has one of the meanings listed below:

- X<sup>21</sup>: D-Pen;  
X<sup>23</sup>: Arg, Nle, Cit, Hci;  
X<sup>24</sup>: Phe, 1-Nal, 2-Nal, Cha;

X<sup>25</sup>: Thi;  
X<sup>28</sup>: Nle, Cha;  
X<sup>29</sup>: Pen;  
X<sup>30</sup>: Trp-NH<sub>2</sub>.

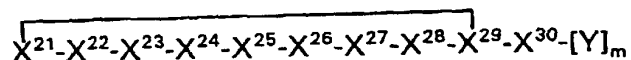
5

Y is a spacer group, for example a peptidic spacer group composed of one or more amino acids, for example poly-Lys, or another spacer group, for example a polyethylene glycol group. The peptide may be coupled to support substances via the group Y.

The peptides of the invention are cyclic peptides having a nine-membered ring, where at least 2, preferably at least 3 and particularly preferably at least 4, of the amino acid residues forming the ring have a sequence from the uPA region 22 to 28.

The present invention further relates to compounds of the general structural formula (I):

20



wherein

X<sup>21</sup>-X<sup>30</sup> are monomeric building blocks, preferably aminocarboxylic acid residues and are derived from a structure in which X<sup>21</sup> = D-Cys, X<sup>22</sup> = Asn, X<sup>23</sup> = Lys, X<sup>24</sup> = Tyr, X<sup>25</sup> = Phe, X<sup>26</sup> = Ser, X<sup>27</sup> = Asn, X<sup>28</sup> = Ile, X<sup>29</sup> = Cys and X<sup>30</sup> = Trp,

Y is a spacer and m is 0 or 1, and

30

the monomeric building blocks are linked via -CONR<sup>1</sup> or -NR<sup>1</sup>CO bonds, in which R<sup>1</sup> in each case independently is hydrogen, methyl or ethyl, and to pharmaceutically acceptable salts and derivatives thereof,

35

with the proviso that at least one of the amino acid residues X<sup>21</sup>-X<sup>30</sup> of the lead structure is replaced by a non-proteinogenic amino acid residue, with the

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resulting compounds preferably having increased protease stability, in particular increased stability against physiological proteases, for example proteases present in blood or tissue, such as, for example, plasmin, or/and proteases present in the digestive tract such as, for example, pepsin, trypsin or chymotrypsin, compared with the lead structure. Preferably, at least the amino acid residue Lys<sup>23</sup> is replaced by a non-proteinogenic amino acid, i.e. by a non-genetically encoded amino acid such as, for example, Orn, Dap, Dab, Cit, Hci or Nle.

Suitable uPAR antagonists are, apart from peptides of the structural formula (I), also pharmaceutically acceptable salts and derivatives thereof. Suitable derivatives are in particular those compounds which have modified reactive side chain groups or/and modified N- or C-terminal groups, for example amino or carboxyl groups. Examples of such modifications are acylation, for example acetylation of amino groups, or/and amidation or esterification of carboxyl groups, for example amidation of the C-terminal amino acid. The monomeric building blocks are linked via NR<sup>1</sup>CO or CONR<sup>1</sup> carboxamide bonds, i.e. the direction of the peptide sequence can be reversed (retropeptides). R<sup>1</sup> may be hydrogen, as in native polypeptides. On the other hand, however, R<sup>1</sup> may also be an alkyl radical, for example methyl or ethyl, and in particular methyl, since N-alkylation of the amide bond can often have a strong effect on the activity (cf. e.g. Levian-Teitelbaum et al., Biopolymers 28 (1989), 51-64). Unless stated otherwise, the  $\alpha$ -aminocarboxylic acids are employed as monomeric building blocks in the form of L-enantiomers.

The peptides of the invention are cyclic compounds, with the monomeric building blocks X<sup>21</sup> and X<sup>29</sup> being bridged with one another. Said bridging may take place, for example, via the side chains of the particular  $\alpha$ -aminocarboxylic acid residues and bridging via



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disulfide bonds, for example between two cysteine residues, is particularly preferred. However, other types of cyclization between amino acid side chains are also possible, for example amide bonds between an amino acid with an amino side group, for example ornithine or Lys, and an amino acid with a carboxyl side chain such as, for example, Asp or Glu. Furthermore, the disulfide bridge may also be replaced by an alkylene bridge, in order to increase chemical stability. Moreover, linkages of an amino acid side chain to the peptide backbone, for example linkage of an amino side group, for example an  $\omega$ -amino side chain, to the C-terminal end, and linkage of a carboxyl side group to the N-terminal end are also possible. A linkage of N- and C-terminus is also possible. The peptides of the invention are obtainable by chemical synthesis, as illustrated in the examples.

Furthermore, the present invention relates to a pharmaceutical composition which contains as active substance at least one peptide or polypeptide as defined above, where appropriate together with pharmaceutically common carriers, excipients or diluents. The peptides or polypeptides of the invention are in particular used for preparing uPAR antagonists which are also suitable for controlling of disorders associated with uPAR expression, in particular for controlling tumors. Furthermore, the peptides of the invention, as well as the lead structure cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]-uPA<sub>21-30</sub>, can be employed as inhibitors of angiogenesis.

The present invention further relates to the use of the uPAR peptide antagonists of the invention for preparing targeting vehicles, for example liposomes, viral vectors, etc., for cells expressing uPAR. Targeting may be carried out for diagnostic applications for controlled transport of labeling groups, for example radioactive or nonradioactive labeling groups.

On the other hand, targeting may be carried out for therapeutic applications, for example for transporting pharmaceutical active substances, for example also for transporting nucleic acids for gene therapy.

5

The pharmaceutical compositions of the invention may be present in any form, for example as tablets, as coated tablets or in the form of solutions or suspensions in aqueous or nonaqueous solvents. The peptides are administered preferably orally or parenterally in liquid or solid form. The preferred carrier medium used for administration in liquid form is water which, where appropriate, contains stabilizers, solubilizers or/and buffers commonly used for injection solutions. Such additives are, for example, tartrate or borate buffer, ethanol, dimethyl sulfoxide, complexers such as, for example, EDTA, polymers such as, for example, liquid polyethylene oxide, etc.

20 Examples of solid carrier substances which may be employed for administration in solid form are starch, lactose, mannitol, methylcellulose, talc, highly dispersed silicon oxide, high molecular weight fatty acids such as, for example, stearic acid, gelatin, agar, calcium phosphate, magnesium stearate, animal and plant fats or solid, high molecular weight polymers such as, for example, polyethylene glycols. Furthermore, the formulations for oral application may also contain flavorings and sweeteners, if desired.

30

The therapeutic compositions of the invention may also be present in the form of complexes, for example with cyclodextrins such as, for example,  $\gamma$ -cyclodextrin. The dose administered depends on the age, state of health and weight of the patient, on the type and seriousness of the disease, on the type of treatment, on the frequency of administration and the type of desired action. The daily dose of the active compound is usually 0.1 to 50 mg/kilogram of body weight.

Typically, 0.5 to 40 and preferably 1.0 to 20 mg/kg/day in one or more doses are sufficient in order to achieve the desired effects.

- 5 The examples and figures described below are intended to further illustrate the invention.

In this connection,

- 10 Figure 1 shows the comparison of the affinities of peptides cyclo[21,29][Cys<sup>21,29</sup>]-uPA<sub>21-30</sub> and cyclo[21-29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]-uPa<sub>21-30</sub> (a) and cyclo[21,29][Cys<sup>21,29</sup>]uPa<sub>21-30</sub> and the corresponding peptide amide (b),  
15 respectively;

Figure 2 shows IC<sub>50</sub> values of modifications of the lead structure cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]uPA<sub>21-30</sub>;

- 20 Figure 3 shows a diagrammatic representation of preferred lead structure modifications;

- Figure 4 shows the stability of the peptides cyclo[19,31]-uPA<sub>16-32</sub>, cyclo[21,29][D-Cys<sup>21</sup>,Tic<sup>25</sup>,Cys<sup>29</sup>]-uPA<sub>21-30</sub> and cyclo[21,29]-  
25 [D-Cys<sup>21</sup>,Cys<sup>29</sup>]uPA<sub>21-30</sub> in human serum (a) and heparinized human blood (b);

- Figure 5 shows the plasmin resistance of uPA peptides  
30 after substitution of Lys<sup>23</sup> by non-proteinogenic amino acids.

## Examples

### 35 1. Methods

#### 1.1 Solid phase peptide synthesis



a disulfide bridge. The oxidation required for this was carried out by taking up 0.1 to 0.3 mg/ml purified linear peptides in 80% water and 20% DMSO (v/v) and removing the solvent under reduced pressure after 10 h. 5 The cyclic peptides were then again purified by HPLC as described before.

### 1.2 Mass spectrometry and amino acid analysis

10 The purified and desalted peptides were analyzed on the HPLC system 140 B (Applied Biosystems, Foster City, USA). UV absorption was measured at 206 nm using a UVIS 200 (Linear Instruments, Reno, USA) detector. The chromatography was carried out on an Aquapore 3  $\mu$  15 (Applied Biosystems, Foster City, USA) reverse phase column (1 mm  $\times$  50 mm) with a flow rate of 20  $\mu$ l/min. The solvent system was 0.1% TFA in water (A) and 0.1% TFA in acetonitrile (B). The HPLC system was coupled to an atmospheric pressure ionization source which was 20 connected to an API III tandem quadrupole instrument (Sciex, Perkin Elmer, Thornhill, Canada).

The quadrupole m/z scale was calibrated using the ammonium adducts of polypropylene glycol. The average 25 masses were calculated from the m/z peaks in the charge distribution profiles of the multiply charged ions (Covey et al., Rapid Commun. Mass Spectrom. 2 (1988), 249-256; Fenn et al., Science 246 (1989), 64-71).

30 The amino acid analysis was carried out according to the ninhydrin method using the analysis system 6300 (Beckman Instruments, Fullerton, USA), after hydrolyzing the peptides by the TFA-HCl vapor-phase method (Tsugita et al., J. Biochem. 102 (1987), 1593- 35 1597), which allows quantitative determination of the peptide concentration.

### 1.3 Flow cytometry

The capacity of the synthetic peptides for inhibiting uPA/uPAR interaction was determined using the human promyeloid cell line U937 as source for cellular native uPAR by means of flow cytometry on a FACScan flow  
5 cytometer (Becton-Dickinson, Heidelberg, Germany) (Chuchulowski et al., Fibrinolysis 6, Suppl. 4 (1992), 95-102; Magdolen et al., (1996), supra). The U937 cells were stimulated with 1 mM phorbol 12-myristate 13-acetate (PMA) for 48 h. After stimulation with PMA,  
10 the U937 cells express considerable amounts of cell surface-associated uPAR.

The stimulated cells were treated with 50 mM glycine-HCl, 0.1 NaCl, pH 3.6 at room temperature for 1 min, in  
15 order to dissociate endogenous receptor-bound uPA. Subsequently, the acidic buffer was neutralized by 0.5 M HEPES, 100 mM NaCl, pH 7.5. The cells were then immediately washed twice with PBS/0.1% bovine serum albumin (BSA) and centrifuged at  $300 \times g$  at room  
20 temperature for 10 min. The cells were resuspended in PBS/0.1% BSA, adjusted to a concentration of  $10^6$  cells per ml and incubated simultaneously with 16 ng of FITC-conjugated pro-uPA and different amounts of the synthetic peptides at room temperature for 45 min.  
25 Prior to analysis, propidium iodide, a fluorescent dye binding specifically double-stranded DNA, was added to each sample, in order to determine the viability of the analyzed U937 cells. Damaged, propidium iodide-labeled cells were excluded from the analysis.

30

#### **1.4 Solid phase uPAR/uPA binding test**

In addition to the analyses by flow cytometry, a solid phase ATF ligand binding test was carried out to  
35 determine the interactions of synthetic peptides with uPAR. For this, microtiter plates were coated with recombinant human uPAR from CHO cells (Wilhelm et al., FEBS Lett. 337 (1994), 131-134; Magdolen et al., Elektrophoresis 16 (1995), 813-816) and the remaining

protein-binding sites were saturated with 2% BSA (w/v). After incubation with the samples (0.6 ng of ATF together with 15  $\mu$ g of synthetic peptide per ml) and two or more washing steps, the amount of ATF which had bound to uPAR immobilized on the microtiter plate was determined using a biotinylated monoclonal mouse antibody against the ATF ring domain (No. 377, American Diagnostica, Greenwich, CT, USA) and subsequently adding avidine-peroxidase conjugate and 3,3',5,5'-tetramethylbenzidine/H<sub>2</sub>O<sub>2</sub> as peroxidase substrate. The presence of synthetic peptides competing with ATF binding to uPAR reduces conversion of the chromogenic substrate.

#### 1.5 Determination of peptide stability in body fluids

The stability of uPA-derived peptides in human serum or complete blood was assayed in vitro.

Human sera were prepared by allowing venous blood to coagulate without anti-coagulant in polypropylene tubes at 37°C for 45 min. The clots adhering to the vessel wall were removed using a plastic stick and removed by centrifugation at 1200  $\times$  g at room temperature for 12 min. The serum in the supernatant was removed and either used freshly for stability studies or frozen in aliquots at -20°C for further use.

For stability studies in human complete blood, coagulation of venous blood was inhibited by heparin sodium (1 000 IU per 10 ml of complete blood), and the blood was used freshly. The peptides to be tested were added to the body fluids in the form of peptide-mixture stock solutions ( $\geq$  1  $\mu$ g per peptide in H<sub>2</sub>O) at a concentration of 5  $\mu$ g/100  $\mu$ l of serum or 200  $\mu$ l of complete blood (per peptide) and incubated at 37°C for various times. Prior to HPLC analysis, complete blood incubations were centrifuged at 16 000  $\times$  g at room

temperature for 5 min and the plasma in the supernatant was removed.

Sera and plasmas were prepurified via HLB precolumns (Waters GmbH, Oasis HLB extraction cartridge 1 cm<sup>3</sup>/30 mg) prior to HPLC analysis. For this purpose, 100 µl of liquid were diluted with PBS to 1 ml and applied to said HLB precolumns equilibrated with 1 ml of 100% methanol and 1 ml of H<sub>2</sub>O. The columns were washed with 1 ml of 5% methanol in H<sub>2</sub>O and eluted with 1 ml of 100% methanol. The first 200 µl (4 drops) of the eluate were discarded as void volume. The next 500 µl of eluate were diluted with 500 µl of PBS and analyzed by HPLC. The remaining 300 µl of the eluate were discarded. HPLC analysis was carried out using a YMC-5 C<sub>18</sub> analytical column and a 20-60% gradient of H<sub>2</sub>O, 0.1% trifluoroacetic acid and acetonitrile, 0.1% trifluoroacetic acid over 30 min and detecting the analytes at 220 nm.

#### 1.6 Determination of protease resistance

The sensitivity of peptides against the attack of various proteases of the digestive tract was determined in vitro using purified enzymes under suitable buffer conditions. In general, 10 µg of peptide were incubated in a 100 µl volume with 2 µg of protease at 37°C for 30 min under the buffer conditions for the activity controls as stated by the manufacturer. After incubation and dilution with 300 µl of H<sub>2</sub>O, the mixtures were analyzed directly, without prepurification via a precolumn, by HPLC using a 20-60% gradient of H<sub>2</sub>O, 0.1% trifluoroacetic acid and acetonitrile, 0.1% trifluoroacetic acid.

Pepsin (Sigma, Deisenhofen, Germany) was incubated with peptides in 52 mM HCl. Incubations with trypsin (Sigma) were carried out in 63 mM sodium phosphate pH 7.6. Chymotrypsins α, β, γ, δ (ICN) were incubated in 50 mM



CaCl<sub>2</sub> and 40 mM Tris/HCl pH 7.8. This was followed by incubating with bacteriae proteinase K in unbuffered water. The positive control used was the peptide cyclo[19,31]-uPA<sub>16-32</sub> which corresponds to the original  
5 sequence of the uPA omega loop except for the cysteine bridge. The peptide sensitivity against the tissue protease plasmin (Sigma) was assayed in mixtures with 10 or 5 µg of peptides and 0.05 U of plasmin in 100 µl of 200 mM sodium phosphate pH 7.5 at 37°C and a 30 min  
10 incubation period.

## 2. Results

### 2.1 Inhibitory action of the peptide cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]uPA<sub>21-30</sub>

15

Figure 1a depicts the inhibitory action of the peptide cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]uPA<sub>21-30</sub> compared with the cyclic peptide cyclo[21,29][Cys<sup>21,29</sup>]-uPA<sub>21-30</sub> which  
20 consists exclusively of L-amino acids. The IC<sub>50</sub> of the cyclic peptide with D-Cys at position 21 was determined to 78 nM, while the IC<sub>50</sub> of the cyclic peptide composed of only L-amino acids was determined to 2 260 nM. In comparison therewith, the IC<sub>50</sub> of the amino-terminal  
25 fragment of uPA (uPA amino acids 1 to 135) is 21 nM.

### 2.2 Synthesis of modified uPA peptides

Using cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]uPA<sub>21-30</sub> as lead  
30 structure, further cyclic peptides were prepared, in which particular amino acids were substituted by other, in particular non-proteinogenic amino acids. The relative activities compared with the lead structure are depicted in figures 1b and 2.

35

Figure 3 depicts examples of particularly preferred modifications of the lead structure.

### 2.3 Results of the studies on peptide stability in human serum and complete blood

The stability of peptides cyclo[19,31]-uPA<sub>16-32</sub> (A),  
5 cyclo[21,29][D-Cys<sup>21</sup>,Tic<sup>25</sup>,Cys<sup>29</sup>]-uPA<sub>21-30</sub> (B) and  
cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]-uPA<sub>21-30</sub> (C) in human serum  
and heparinized human blood was assayed. Figures 4a and  
4b depict the results of these experiments. The peaks  
at 20.5 min correspond to peptide A, the peaks at 23.0  
10 min correspond to peptide B and the peaks at 24.9 min  
correspond to peptide C.

The stability of the peptides in human serum (fig. 4a) was studied by adding a mixture of in each case 12.5  $\mu$ g of the peptides to about 250  $\mu$ l of serum. 100  $\mu$ l thereof were diluted with PBS to 1 ml, prepurified on an HLB precolumn and analyzed immediately by HPLC (middle profile). A further 100  $\mu$ l were analyzed after 20.5 h of incubation at 37°C (bottom profile). As a control, in each case 5  $\mu$ g of the peptides were admixed with 1 ml of PBS and analyzed (top profile). The peak at 21.2 min corresponds to a nonidentified metabolite.

The stability in heparinized human blood (figure 4b) was studied by adding 750  $\mu$ l of freshly prepared heparinized human blood to 37.5  $\mu$ g of the peptides. Immediately thereafter, 375  $\mu$ l were removed by centrifugation. 100  $\mu$ l of the plasma supernatant were analyzed (middle profile). The remaining 375  $\mu$ l were incubated with gentle agitation at 37°C for 20.5 h and then analyzed (bottom profile). As a control, 5  $\mu$ g of the peptides had already been admixed with 1 ml of PBS and analyzed (top profile).

35 After incubation in human serum at 37°C for 20.5 h, peptide A (cyclo[19,31]-uPA<sub>16-32</sub>) could no longer be detected. Instead a new peak with relatively longer retention time (fig. 4a, bottom HPLC profile, RT 21.233 min) appeared, which corresponds presumably to a

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metabolite of A. In contrast, the relative retention times and peak integrals of peptides B (cyclo[21,29][D-Cys<sup>21</sup>Tic<sup>45</sup>Cys<sup>29</sup>]-uPA<sub>21-30</sub>) and C (cyclo[21,29][D-Cys<sup>21</sup>Cys<sup>29</sup>]-uPA<sub>21-30</sub>) remained nearly the same, after exposure to human serum. This allows the conclusion to be reached that the chemical identity and concentration of the two peptides are unaltered after a 20.5-hour exposure to human serum.

Exposing the peptides to freshly isolated heparinized complete blood over 20.5 h, too, showed the instability of A. In this case, it was not possible to detect either the unaltered substance or a suspected metabolite. In contrast, the peaks of peptides B and C appeared stable in human blood. Compared with the PBS control, the A peak appears already substantially reduced in the sample worked up and analyzed immediately after addition to complete blood. Apparently, the short period between addition of the peptides and prepurification on precolumns (10-15 min) was sufficient to degrade a substantial part of the amount of peptide added (> 80%).

The peak integrals of the two D-Cys derivatives B and C from plasma are distinctly larger than in the PBS control, although they were used at the same concentration, based on total volumes of PBS and complete blood. After incubation, however, the peptides were analyzed only in the plasma after removing the blood cells. This may be regarded as an indication that the D-Cys derivatives are distributed mainly in the plasma but are unable to penetrate or bind blood cells in a significant manner.

#### 2.4 Stability of uPA peptides against plasmin

The peptide lead structure cyclo[21,29][D-Cys<sup>21</sup>Cys<sup>29</sup>]-uPA<sub>21-30</sub> and modifications thereof at position 23, cyclo[21,29][D-Cys<sup>21</sup>Orn<sup>23</sup>Cys<sup>29</sup>]-uPA<sub>21-30</sub> (ornithine),



a new peak appeared after incubation with plasmin, whose retention time was nearly identical to that of the plasmin metabolite of the unaltered lead structure, i.e. the Arg<sup>23</sup>-substituted variant is plasmin-sensitive.

5 When exposing the diaminopropionic acid-substituted variant to plasmin, two small unidentified peaks appeared at approx. 21 min, whose retention time differs greatly from the retention times of the plasmin metabolites of the lead structure and those of the Arg<sup>23</sup>  
10 variant. Correspondingly, it is questionable whether the small 21 min peaks indeed represent specific plasmin cleavage products of the Dap<sup>23</sup> variant.

Substitution of lysine at position 23 of the  
15 cyclo[21,29][D-Cys<sup>21</sup>Cys<sup>29</sup>]-uPA<sub>21-30</sub> lead structure by non-proteinogenic amino acids may generate stability against the tissue protease plasmin, without substantially altering the biological activity.

## 20 **2.5 Anti-angiogenetic effectiveness**

Thorax aortae were obtained from 1- to 2-month-old Wistar rats and immediately transferred into a culture dish containing serum-free medium (RPMI). The tissue  
25 surrounding the aorta was carefully removed. Aorta rings of 1 mm in length were prepared and thoroughly washed with serum-free medium. Before embedding the aorta rings in Matrigel, the bottom of each well was coated with 80 µl of gel solution. After gel formation,  
30 the aorta rings were transferred into the well, positioned and fixed by overlaying with 70 µl of gel solution. After gel formation, various amounts of the particular test peptide were introduced into the wells. Controls studied were medium alone, medium with growth  
35 supplements and medium with growth supplements and control peptide. The cultures were kept at 35°C for 5 days and then studied.

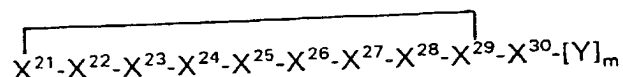
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The test peptides were cyclo[21,29][D-Cys<sup>21</sup>,Cys<sup>29</sup>]-uPA<sub>21-30</sub> and cyclo[21,29][D-Cys<sup>21</sup>,Nle<sup>23</sup>Cys<sup>29</sup>]-uPA<sub>21-30</sub> in dosages of 0.001, 0.1, 1, 10, 25 and 50 µg/ml. The test peptides displayed distinct anti-angiogenetic actions in the in-vitro tissue culture assay. Capillary formation was found to be inhibited in the concentration range of about 1 µg/ml and higher. Capillary budding, i.e. the number and length of newly formed capillaries, could be reduced at a peptide concentration of 30 µg/ml by a factor of about 3 to 4, compared with inactive control peptides.

Claims

5

1. A compound of the general structural formula (I):



- 10 wherein  $X^{21}-X^{30}$  are monomeric building blocks, preferably aminocarboxylic acid residues and are derived from a structure in which  $X^{21}$  = D-Cys,  $X^{22}$  = Asn,  $X^{23}$  = Lys,  $X^{24}$  = Tyr,  $X^{25}$  = Phe,  $X^{26}$  = Ser,  $X^{27}$  = Asn,  $X^{28}$  = Ile,  $X^{29}$  = Cys and  $X^{30}$  = Trp, Y is a  
15 spacer and m is 0 or 1, and the monomeric building blocks are linked via  $-\text{CONR}^1$  or  $-\text{NR}^1\text{CO}$  bonds, in which  $R^1$  in each case independently is hydrogen, methyl or ethyl, and pharmaceutically acceptable salts and derivatives thereof,  
20 with the proviso that at least one of the amino acid residues  $X^{21}-X^{30}$  of the lead structure is replaced by one of the amino acid residues listed below:

- 25  $X^{21}$ : Asp, Glu, 2,3-diaminopropionic acid (Dap),  
2,4-diaminobutyric acid (Dab),  
D-penicillanine (D-Pen), allylglycine (Alg),  
ornithine (Orn), Lys;  
 $X^{22}$ : Asp, Glu;  
30  $X^{23}$ : Dab, Dap, His, citrulline (Cit),  
homocitrulline (Hci), norleucine (Nle);  
 $X^{24}$ : homophenylalanine (Hph), 1,2,3,4-  
tetrahydroisoquinoline-3-carboxylic acid  
(Tic), thienylalanine (Thi), Trp,  
35 phenylglycin (Phg), 1-naphthylalanine (1-  
Nal), 2-naphthylalanine (2-Nal), Cha  
(cyclohexylalanine);  
 $X^{25}$ : Trp, Tic, Thi, Hph, Phg;

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X<sup>26</sup>: Val;

X<sup>27</sup>: Asp, Glu;

X<sup>28</sup>: Cha, 2-aminobutyric acid (Abu), tert-leucine (Tle),  $\alpha$ -aminoisobutyric acid (Aib);

5 X<sup>29</sup>: Asp, Glu, Dap, Dab, Alg, D-Pen, Orn, Lys;

2. The compound as claimed in claim 1,  
**characterized in that**

10 at least one of the amino acid residues X<sup>21</sup>-X<sup>30</sup> of  
the lead structure has one of the meanings listed  
below:

X<sup>21</sup>: D-Pen;

X<sup>23</sup>: Dap, Dab, Cit, Hci, Nle, His;

X<sup>24</sup>: Thi, Hph, Phg, 1-Nal, 2-Nal, Cha;

15 X<sup>25</sup>: Thi;

X<sup>27</sup>: Asp;

X<sup>28</sup>: Val, Cha.

3. The compound as claimed in claim 1,  
**characterized in that**

20 at least one of the amino acid residues X<sup>21</sup>-X<sup>30</sup> of  
the lead structure has one of the meanings listed  
below:

X<sup>21</sup>: D-Pen;

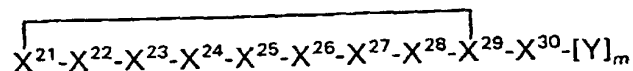
25 X<sup>23</sup>: Dab, Nle, Cit, Hci;

X<sup>24</sup>: 1-Nal, 2-Nal, Cha;

X<sup>25</sup>: Thi;

X<sup>28</sup>: Cha.

30 4. A compound of the general structural formula (I):



35 wherein X<sup>21</sup>-X<sup>30</sup> are monomeric building blocks,  
preferably aminocarboxylic acid residues and are  
derived from a structure in which X<sup>21</sup> = D-Cys, X<sup>22</sup>  
= Asn, X<sup>23</sup> = Dap, Dab or Nle, X<sup>24</sup> = Tyr, X<sup>25</sup> = Phe,



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$X^{26}$  = Ser,  $X^{27}$  = Asn,  $X^{28}$  = Ile,  $X^{29}$  = Cys and  $X^{30}$  = Trp, Y is a spacer and m is 0 or 1, and the monomeric building blocks are linked via  $-\text{CONR}^1$  or  $-\text{NR}^1\text{CO}$  bonds, in which  $R^1$  in each case independently is hydrogen, methyl or ethyl, and pharmaceutically acceptable salts and derivatives thereof.

5. The compound as claimed in any of the preceding claims,

**characterized in that**

at least 2 of the amino acid residues  $X^{22}$ ,  $X^{23}$ ,  $X^{24}$ ,  $X^{25}$ ,  $X^{26}$ ,  $X^{27}$ ,  $X^{28}$  and  $X^{30}$  have the same side chain as an amino acid at the same position in the native uPA sequence.

6. The compound as claimed in claim 5,

**characterized in that**

at least 2 of the amino acid residues  $X^{24}$ ,  $X^{25}$ ,  $X^{28}$  and  $X^{30}$  have the same side chain as in the native uPA sequence.

7. A pharmaceutical composition, which contains as active substance at least one compound as claimed in any of claims 1 to 6, where appropriate together with pharmaceutically common carriers, excipients or diluents.

8. The use of a compound as claimed in any of claims 1 to 6 for preparing a uPAR antagonist.

9. The use as claimed in claim 8 for controlling disorders associated with uPAR expression, in particular for controlling tumors.

10. The use of a compound as claimed in any of claims 1 to 6 for preparing a targeting vehicle for cells expressing uPAR.

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11. The use of a compound as claimed in any of  
claims 1 to 6 for preparing an angiogenesis  
inhibitor.

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**Abstract**

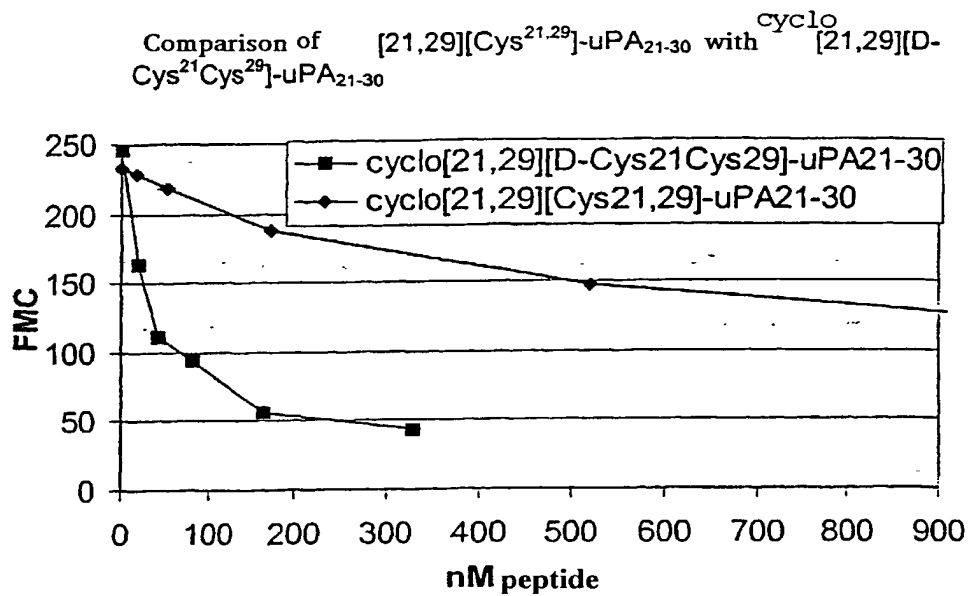
The present invention relates to cyclic peptides as inhibitors of urokinase binding to the urokinase  
5 receptor. Said cyclic peptides are suitable as pharmaceutical active substances for disorders mediated by urokinase and its receptor.

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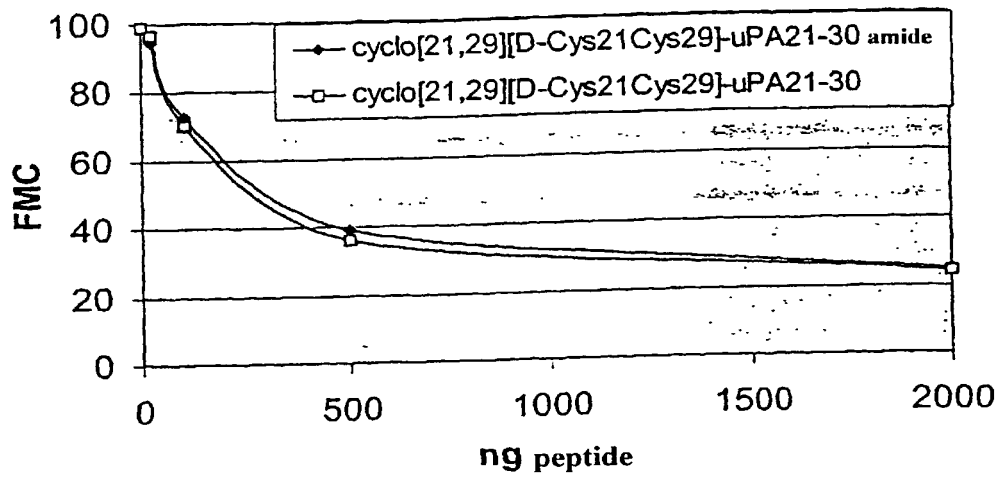
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Fig. 1a



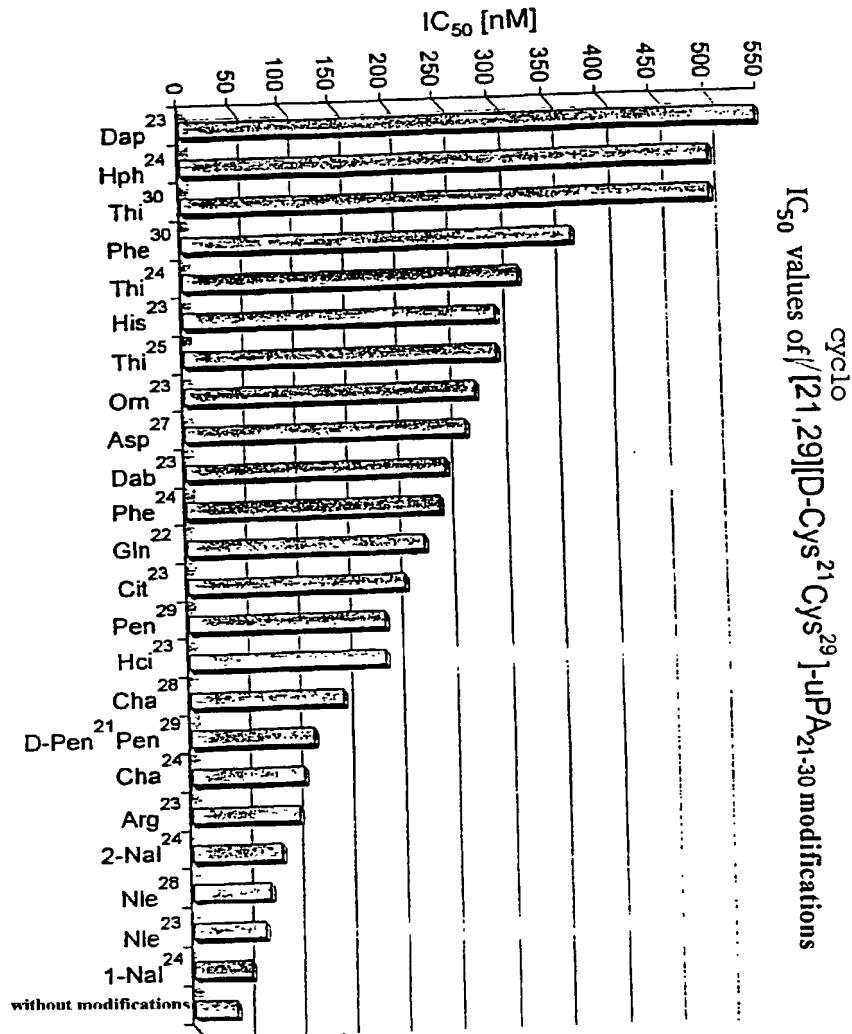
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Fig. 1b

cyclo[21,29][D-Cys<sup>21</sup>Cys<sup>29</sup>]-uPA<sub>21-30</sub> and the corresponding peptide amide

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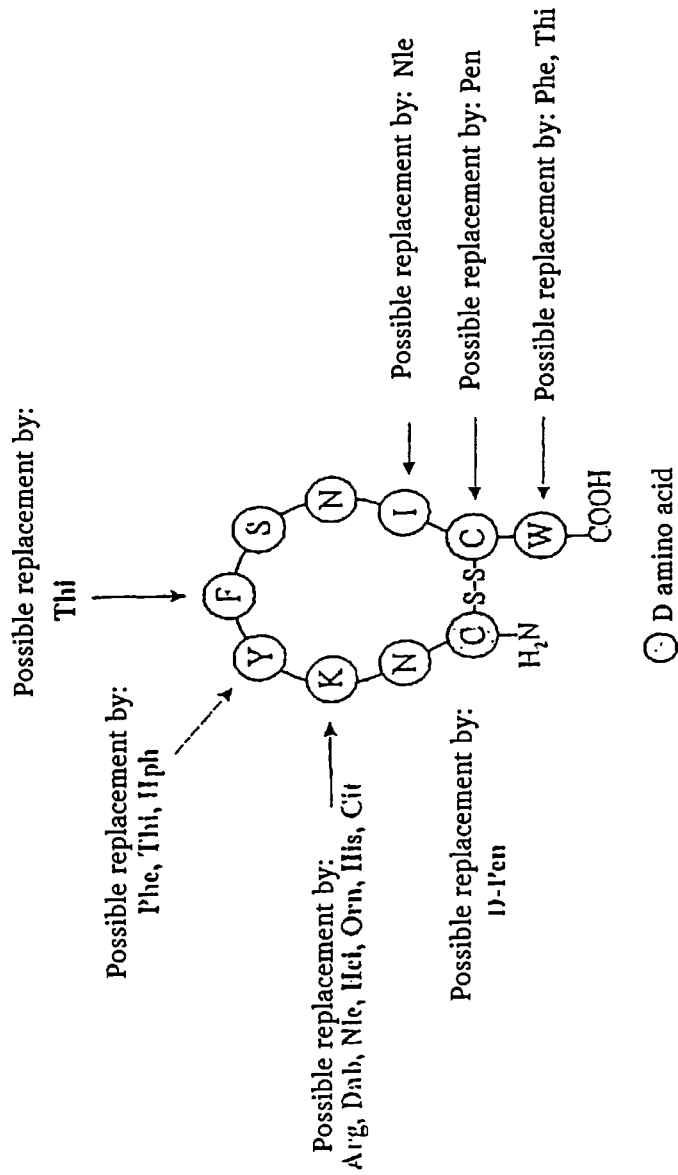
Fig. 2



REPLACEMENT SHEET (RULE 26)

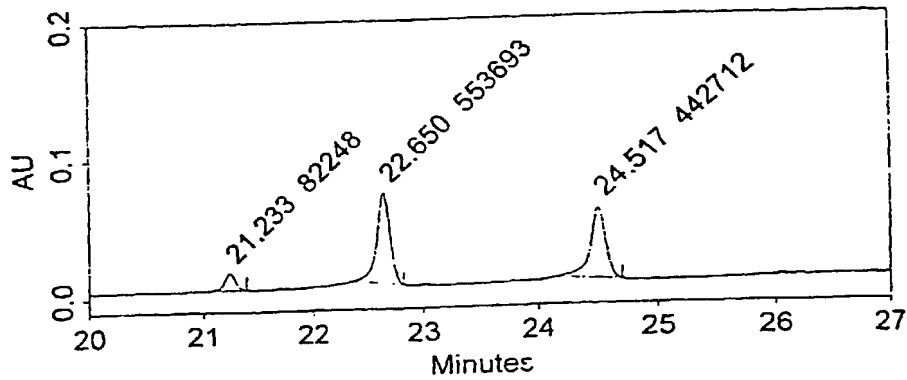
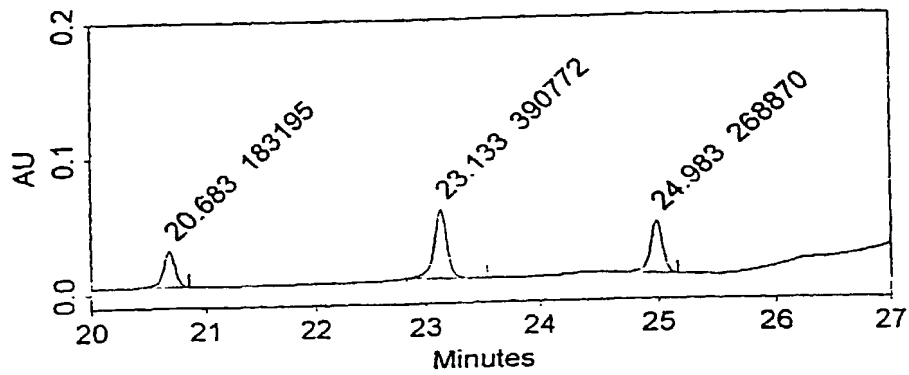
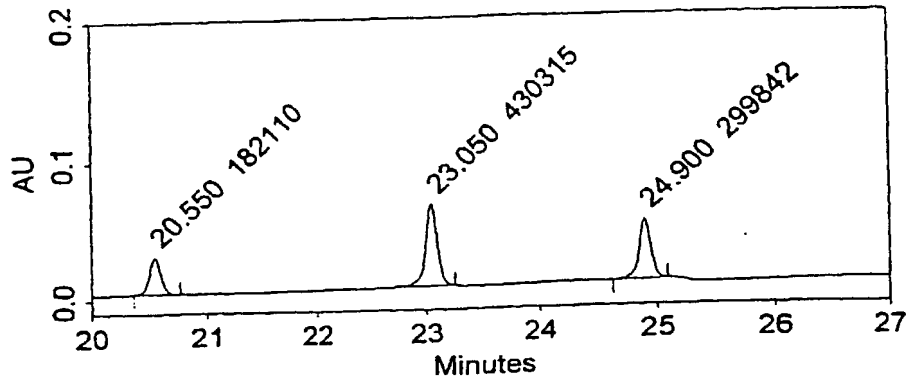
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Fig. 3



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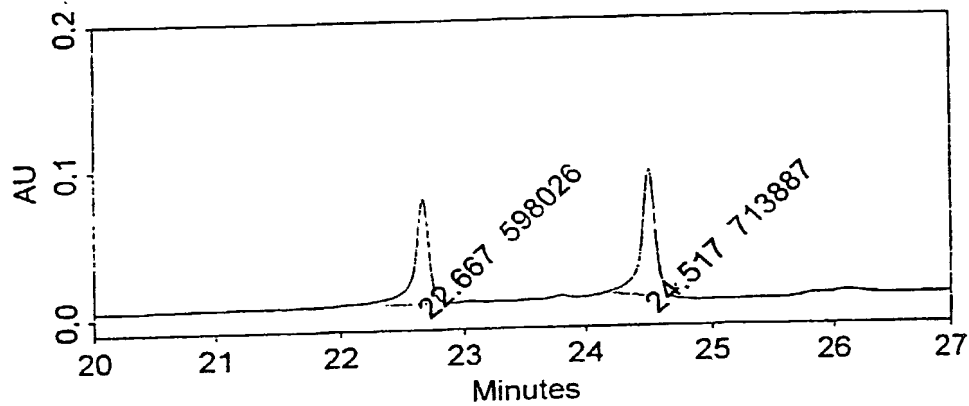
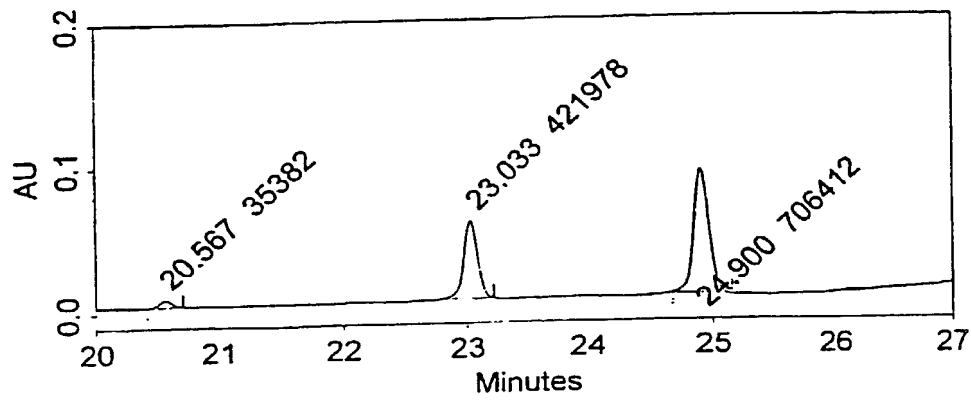
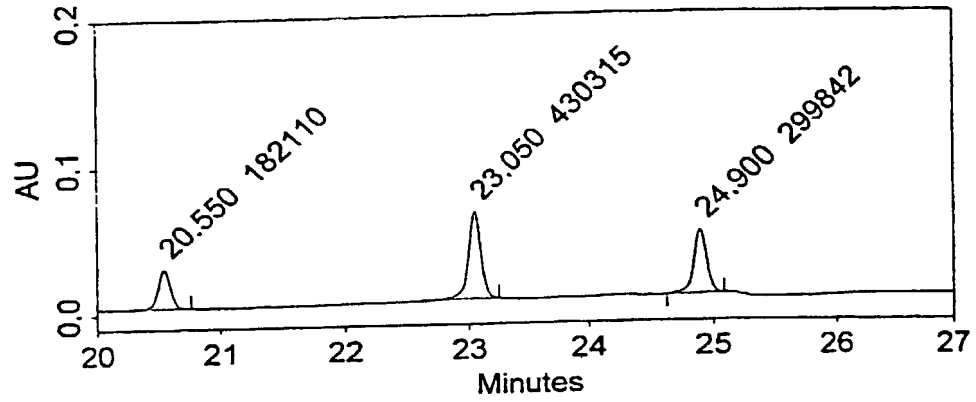
Fig. 4a





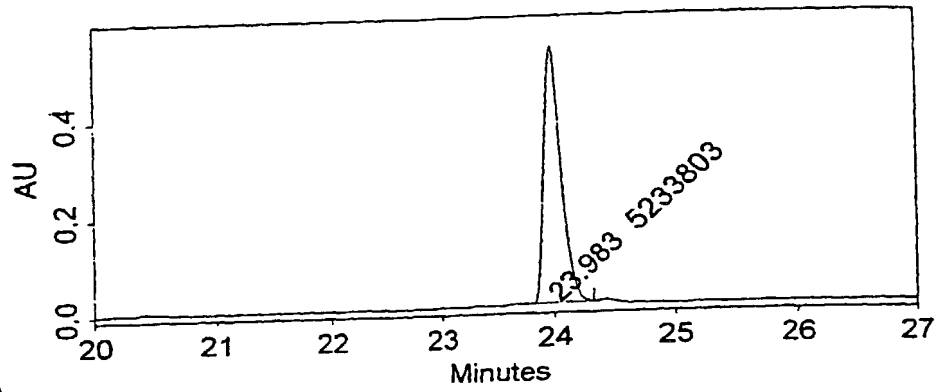
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Fig. 4b

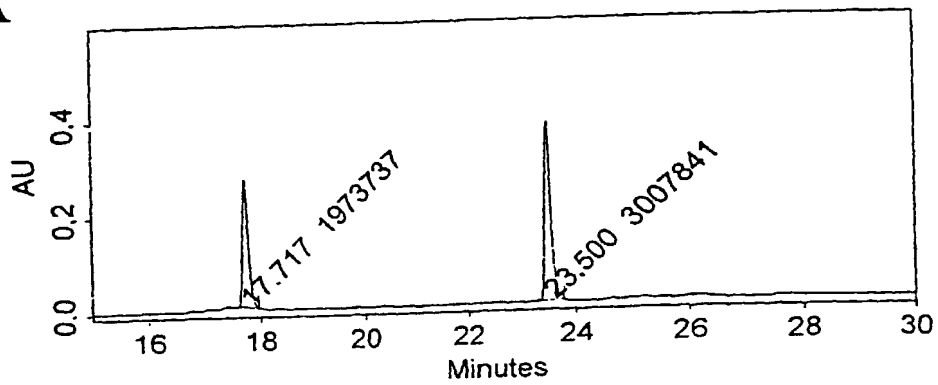


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Fig. 5

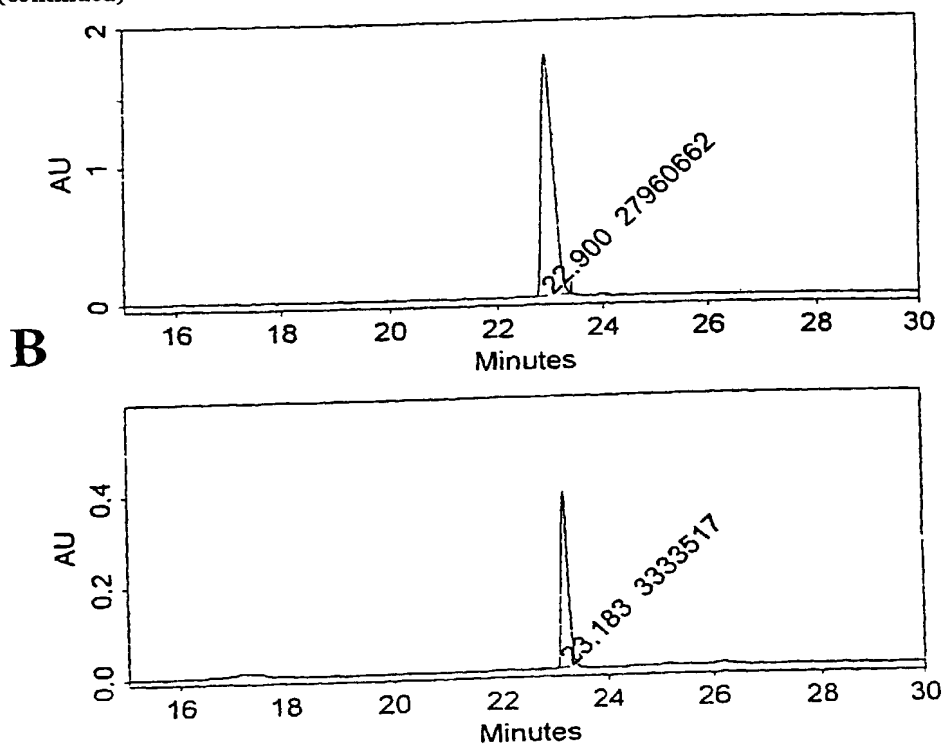


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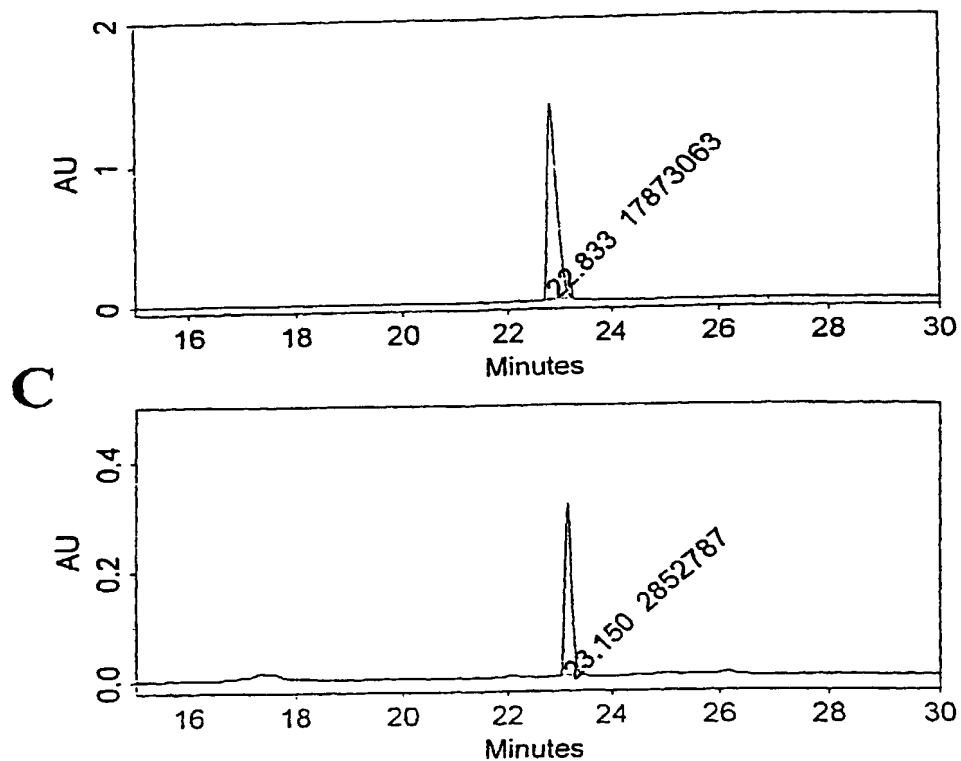
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Fig. 5  
(continued)



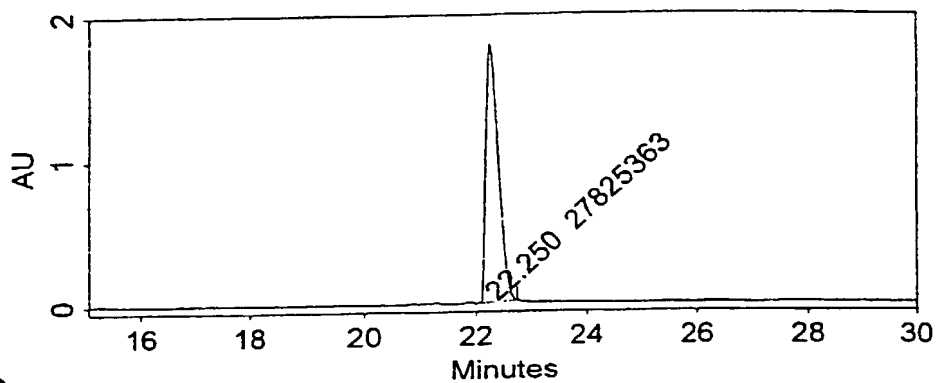
- 9/12 -

Fig. 5  
(continued)

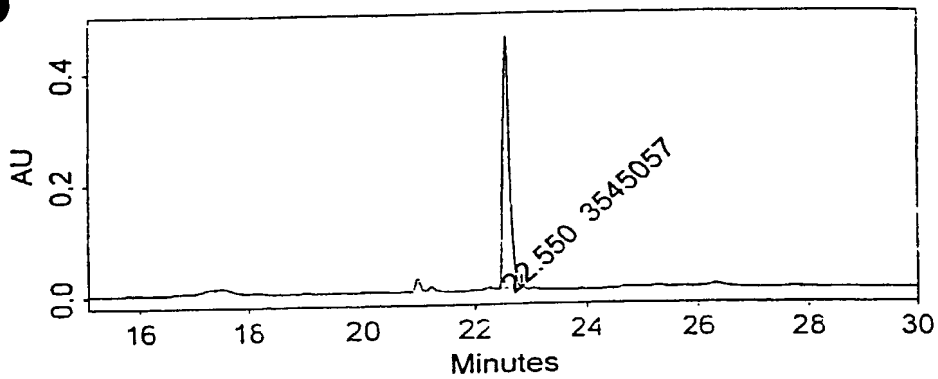


- 10/12 -

Fig. 5  
(continued)

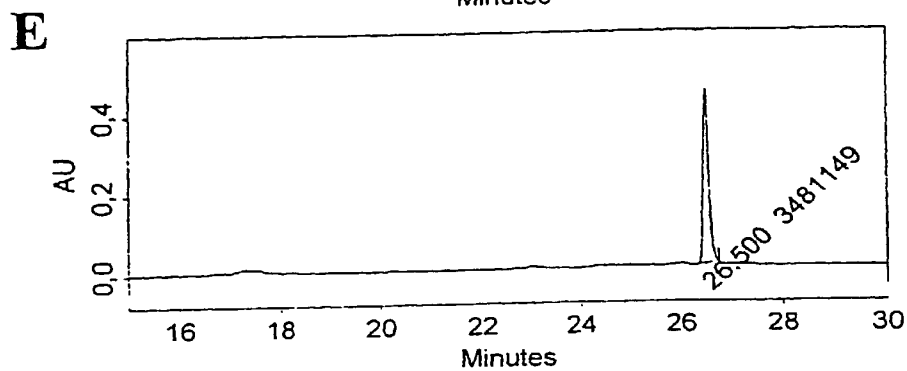
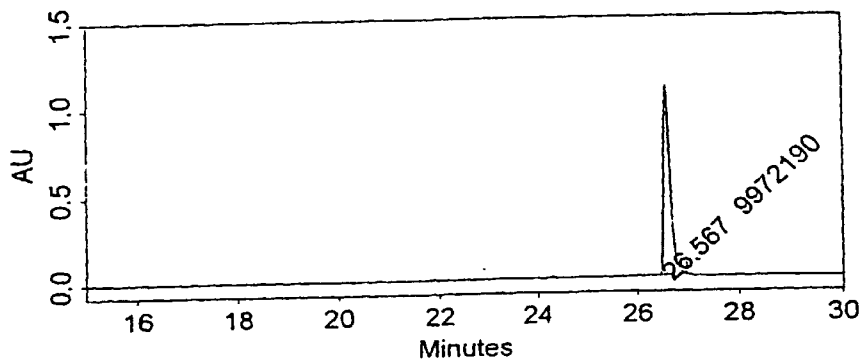


D



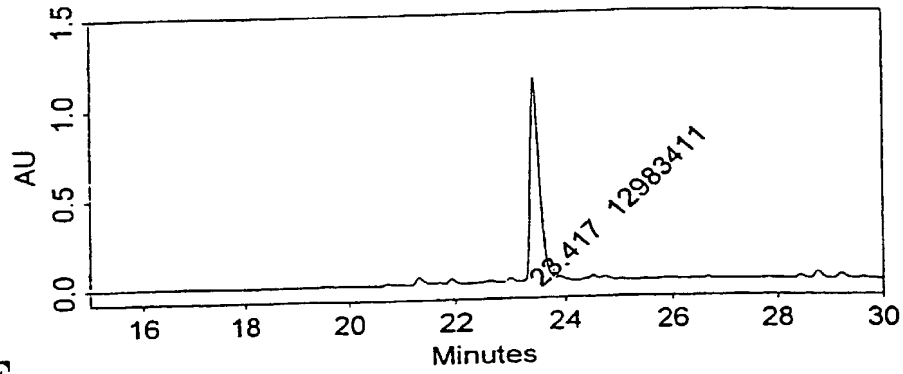
- 11/12 -

Fig. 5  
(continued)

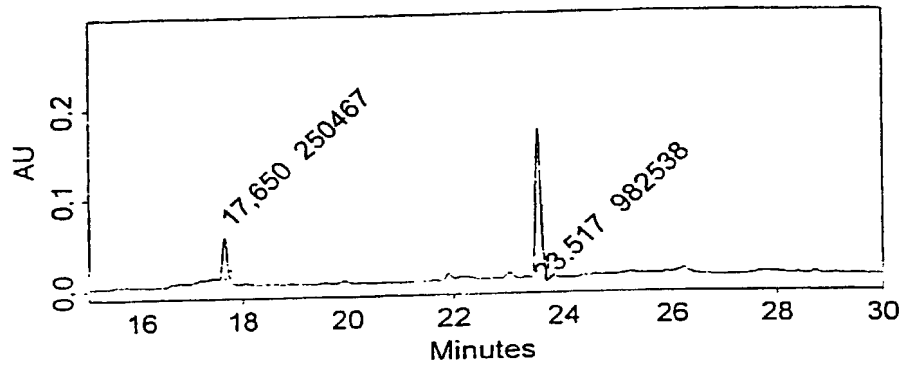


- 12/12 -

Fig. 5  
(continued)



F



Docket No. \_\_\_\_\_

ARENT FOX KINTNER PLOTKIN &amp; KAHN, PLLC

Nikaido, Marmelstein, Murray &amp; Oram Intellectual Property Group

**Declaration For U.S. Patent Application**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

(Insert Title) Cyclic peptidomimetic urokinase receptor antagonists

the specification of which is attached hereto unless the following box is checked:

☒ X was filed on 19 July 2000 as PCT International Application  
 Number PCT/EP00/06905 and was amended on \_\_\_\_\_  
 and/or was filed on \_\_\_\_\_ as United States Application  
 Number \_\_\_\_\_ and was amended on \_\_\_\_\_

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International Application having a filing date before that of the application(s) for which priority is claimed:

|                                                                    |                     |                |                        |                                                                                                                                                                                                                 |
|--------------------------------------------------------------------|---------------------|----------------|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (List prior foreign applications. See note A on back of this page) | <u>199 33 701.2</u> | <u>Germany</u> | <u>19/July/1999</u>    | Priority Claimed<br><input checked="" type="checkbox"/> Yes <input type="checkbox"/> No<br><input type="checkbox"/> Yes <input type="checkbox"/> No<br><input type="checkbox"/> Yes <input type="checkbox"/> No |
|                                                                    | (Number)            | (Country)      | (Day/Month/Year Filed) |                                                                                                                                                                                                                 |
|                                                                    | (Number)            | (Country)      | (Day/Month/Year Filed) |                                                                                                                                                                                                                 |
|                                                                    | (Number)            | (Country)      | (Day/Month/Year Filed) |                                                                                                                                                                                                                 |

I hereby claim the benefit under 35 U.S.C. §119(c) of any United States provisional application(s) listed below.

|                      |               |
|----------------------|---------------|
| (Application Number) | (Filing Date) |
| (Application Number) | (Filing Date) |

(See Note B on back of this page)

☐ See attached list for additional prior foreign or provisional applications.

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) (U.S. or PCT) in the manner provided by the first paragraph of 35, U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

|                                                                                       |                          |               |                                         |
|---------------------------------------------------------------------------------------|--------------------------|---------------|-----------------------------------------|
| (List prior U.S. Applications or PCT International applications designating the U.S.) | (Application Serial No.) | (Filing Date) | (Status) (patented, pending, abandoned) |
|                                                                                       | (Application Serial No.) | (Filing Date) | (Status) (patented, pending, abandoned) |

And I hereby appoint as principal attorneys: Robert B. Murray, Reg. No. 22,980; Charles M. Marmelstein, Reg. No. 25,895; George E. Gram, Jr., Reg. No. 27,931; Douglas H. Goldhush, Reg. No. 33,125; David T. Nikaido, Reg. No. 22,663; Monica Chin Kius, Reg. No. 36,105; Richard J. Berman, Reg. No. 39,107; King L. Wong, Reg. No. 37,500; James A. Poulos, III, Reg. No. 31,714; Patrick D. Muir, Reg. No. 37,403; Murat Ozgu, Reg. No. 44,275; Bradley D. Goldizen, Reg. No. 43,637; N. Alexander Nolte, Reg. No. 45,682 and Robert K. Carpenter, Reg. No. 34,794.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note C on back of this page)

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Post Office Address \_\_\_\_\_

Full name of ninth joint inventor, if any \_\_\_\_\_  
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Residence \_\_\_\_\_ Date \_\_\_\_\_  
Citizenship \_\_\_\_\_  
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